

UNCLASSIFIED

AD NUMBER
ADB218884
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM DISTRIBUTION AUTHORIZED TO U.S. GOV'T. AGENCIES ONLY; PROPRIETARY INFO.; SEP 96. OTHER REQUESTS SHALL BE REFERRED TO COMMANDER, ARMY MEDICAL RESEARCH AND MATERIEL COMMAND, ATTN: MCMR-RMI-S, FORT DETRICK, MD 21702-5012.
AUTHORITY
P. Rhinehart, via mcmr-rmi-s[70-1y] ltr dtd Apr 2, 1998.

THIS PAGE IS UNCLASSIFIED

AD _____

MIPR NO. 94MM4564

TITLE: Human Breast Cancer Cell Proliferation and Modulation by Melatonin and Environmental Magnetic Fields

PRINCIPAL INVESTIGATOR: Robert P. Liburdy, Ph.D.

CONTRACTING ORGANIZATION: Lawrence Berkeley Laboratory
Berkeley, CA 94720

REPORT DATE: September 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Sep 96). Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

DDIC QUALITY INSPECTED 1

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 1996	3. REPORT TYPE AND DATES COVERED Annual (1 Jul 95 - 30 Jun 96)	
4. TITLE AND SUBTITLE Human Breast Cancer Cell Proliferation and Modulation by Melatonin and Environmental Magnetic Fields			5. FUNDING NUMBERS 94MM4564	
6. AUTHOR(S) Robert P. Liburdy, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Lawrence Berkeley Laboratory Berkeley, CA 94720			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick Frederick, Maryland 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES			19970113 039	
12a. DISTRIBUTION / AVAILABILITY STATEMENT Distribution authorized to U.S. Government agencies only; Proprietary Information, Sep 96. Other requests for this document shall be referred to Comander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) The overall purpose of this study is to investigate the role that environmental-level magnetic fields may play as an exogenous factor in the etiology of human breast cancer. There are several important observations that have been made during the research conducted in this reporting period. We have further characterized the EMF blocking effect on the oncostatic action of melatonin and tamoxifen on human breast cancer cell growth in vitro. Field dose studies suggest that this blocking effect is observed at field strengths of up to 1Gauss (1000mG, 100uTesla) with a lower threshold between 6 - 12mG. Studies investigating the critical duration of exposure suggest that at least two days of continuous field exposure is required; this corresponds to one cell-cycle period. In biologically-based studies it was shown that melatonin concentration in the media itself is not influenced by a 2 vs 12mG magnetic field. The original melatonin findings presented in our proposal recently have been independently replicated at the EPA.				
14. SUBJECT TERMS Breast Cancer, Environmental Magnetic Fields, Melatonin, Cell Proliferation, Tamoxifen, Estrogen Receptor			15. NUMBER OF PAGES 25	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT limited	

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Robert P. Liburdy Sep 19 1996
PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION	page 5
BODY	page 6
CONCLUSIONS	page 17
REFERENCES	page 18
APPENDIX	page 19

Introduction.

Please note this report covers the second year of research activity. A no-cost extension has been requested and I will be glad to submit an official "final" report at the end of the no-cost extension time period.

The overall purpose of this study is to investigate the role that environmental-level magnetic fields may play as an exogenous risk factor in the etiology of human breast cancer. We are testing the hypothesis that an environmental-level 12 mGauss 60 Hz magnetic field influences hormone (melatonin) and drug (tamoxifen) interactions with human breast cancer cells. This approach is based on our recent findings that a 12 mGauss (60 Hz, sinusoidal) magnetic field (a) blocks the cytostatic action of melatonin and (b) new findings that the same 12 mGauss magnetic field significantly inhibits the cytostatic action of tamoxifen on MCF-7 breast cancer cell growth. As indicated in our proposal we will undertake biophysically-based (Phase I) and biologically-based (Phase II) in vitro studies to investigate these interactions. Our goal is to understand these interactions from the point of field coupling (biophysical insights) and cellular responses (biological insights).

Phase I: Biophysically-Based Studies.

1. (Y1) Determine the magnetic field dose threshold. We have observed that a 12 mGauss magnetic field blocks melatonin's oncostatic function, but a 2 mGauss field does not. A dose-threshold should exist between 2 - 12 mGauss and we will attempt to identify this threshold.

2. (Y1-Y2) Determine if the exposure metric is either the time-varying magnetic field itself or the induced electric field. Tests based on Faraday's law of current induction will involve rotating the orientation of the magnetic field by 90° to reduce the induced electric field significantly while maintaining the magnetic field intensity.

3. (Y2-Y3) Determine if there is a frequency dependence. Frequency will be varied between 15 - 300 Hz. This covers a 5-fold range of 60 Hz harmonics.

4. (Y3) Determine if there is a threshold for exposure time. We will test if magnetic fields are required during the first 24 hours of hormone/drug interaction with target cells during cell culture. A critical threshold may exist for magnetic field exposure during the cell cycle. Our original exposures were continuous during the eight-day growth period. This is related to Aim 5.

5. (Y4) Determine if the 12 mGauss magnetic field effect is reversible. We will perform a 12 mGauss exposure of MCF-7 cells at the threshold time defined in Aim 4 in the presence of melatonin or tamoxifen, and we will then use these cells to test if they are still responsive to melatonin or tamoxifen in a second growth curve experiment. The second experiment will employ 2 and 12 mGauss magnetic fields, for comparison.

Phase II: Biologically-Based Studies.

6. (Y1-Y2) Determine the dose-response relationship for melatonin and tamoxifen for the 12 mGauss magnetic field effect. We will examine a dose range covering physiological and pharmacological doses of melatonin (10^{-11} to 10^{-5} M) and tamoxifen (10^{-8} to 10^{-6} M), respectively.

7. (Y2) Determine if entry and steady-state levels of melatonin and tamoxifen in MCF-7 cells are altered by the 12 mGauss magnetic field. One way to test this is by following radiolabelled hormone/drug entry into MCF-7 cells.

8. (Y3) Determine if the internal distribution of hormone/drug in the target cell is altered by 12 mGauss magnetic fields. We plan to use quantitative, digital imaging microscopy and antibody-based fluorescent probes at the single-cell level.

9. (Y3-Y4) Determine if there is an indirect magnetic field interaction involving signal transduction (ST) which counteracts the growth inhibition action of melatonin and tamoxifen. Some magnetic fields are reported to alter intracellular calcium levels in cells, and intracellular calcium is also linked to ER expression in MCF-7 cells. We plan to assess intracellular calcium at the single-cell level in 2 and 12 mGauss magnetic fields.

Body.

Experimental Design.

In our cell culture studies we employ the following experimental design shown in **Figure 1**. Cells are plated out in 35mm plates and treated with melatonin or tamoxifen and placed into incubators corresponding to a desired field strength. We have at least four incubators free for such an experiment so that cells of the same passage can be used at the same time in a dose-response experiment in incubators set at different field strengths. We feel that this helps to reduce any "noise" in comparing data across treatment groups associated with any variation among cell passage numbers. We always include a set of plates in each incubator that have no drug or hormone so we can compare growth curves across incubators; these should be identical and we use this information as a quality control check-point. See **Figure 2** which shows that growth curves are not significantly different for cells that were split and grown simultaneously in four incubators.

We have also developed a specialized exposure system to propagate cells for routine passage in a well-defined 2mG, 60Hz magnetic field. Cells are placed inside of a 4-square Merritt coil and this is housed inside of a mu-metal shielding chamber. This quality control effort insures that our cells are maintained in a uniform, low-level magnetic field for routine passage prior to use in any magnetic field studies. **Figure 3** shows this magnetic field exposure system; we monitor temperature (in the vicinity of cell culture plates) and CO₂. The magnetic fields have also been mapped inside of this exposure environment. **Figure 4a** shows the AC magnetic fields inside of a typical commercial incubator without mu-metal shielding; there is a significant variation in field intensity. **Figure 4b** shows the field intensity mapping performed after a mu-metal shielding chamber is placed inside of the incubator with the Merritt coil energized to generate a 12.32 ± 0.03 mG 60Hz magnetic field. The field is reasonably uniform and spatially well-defined.

Phase 1: Biophysical Studies.

Field Dose-Response Studies.

We have continued to address the question of field intensity threshold for the magnetic field blocking effect on melatonin oncostatic action. We have previously conducted a series of experiments at 2, 6, and 12mG, and we have continued such experiments over this dose range. **Figure 5** shows the results of such a series of experiments. I note that these studies were performed as described above so that cells of the same passage were used across incubators. There were eight experiments in which 2 and 12mG were used simultaneously, and four experiments in which 2, 6, and 12mG were used simultaneously. Across these experiments we observe that there appears to be no field blocking effect at 2 or 6mG. At these two field intensities there is an approximate 18% growth inhibition due to melatonin (10^{-9} M). This growth inhibition due to melatonin is significant as shown in the figure. At 12mG we observe no significant difference ($p > 0.05$) between \pm melatonin treatment groups. Thus, the 12mG field blocked the action of melatonin, and the data suggests that a field-dose threshold may exist between 6 and 12mG.

Experimental Design

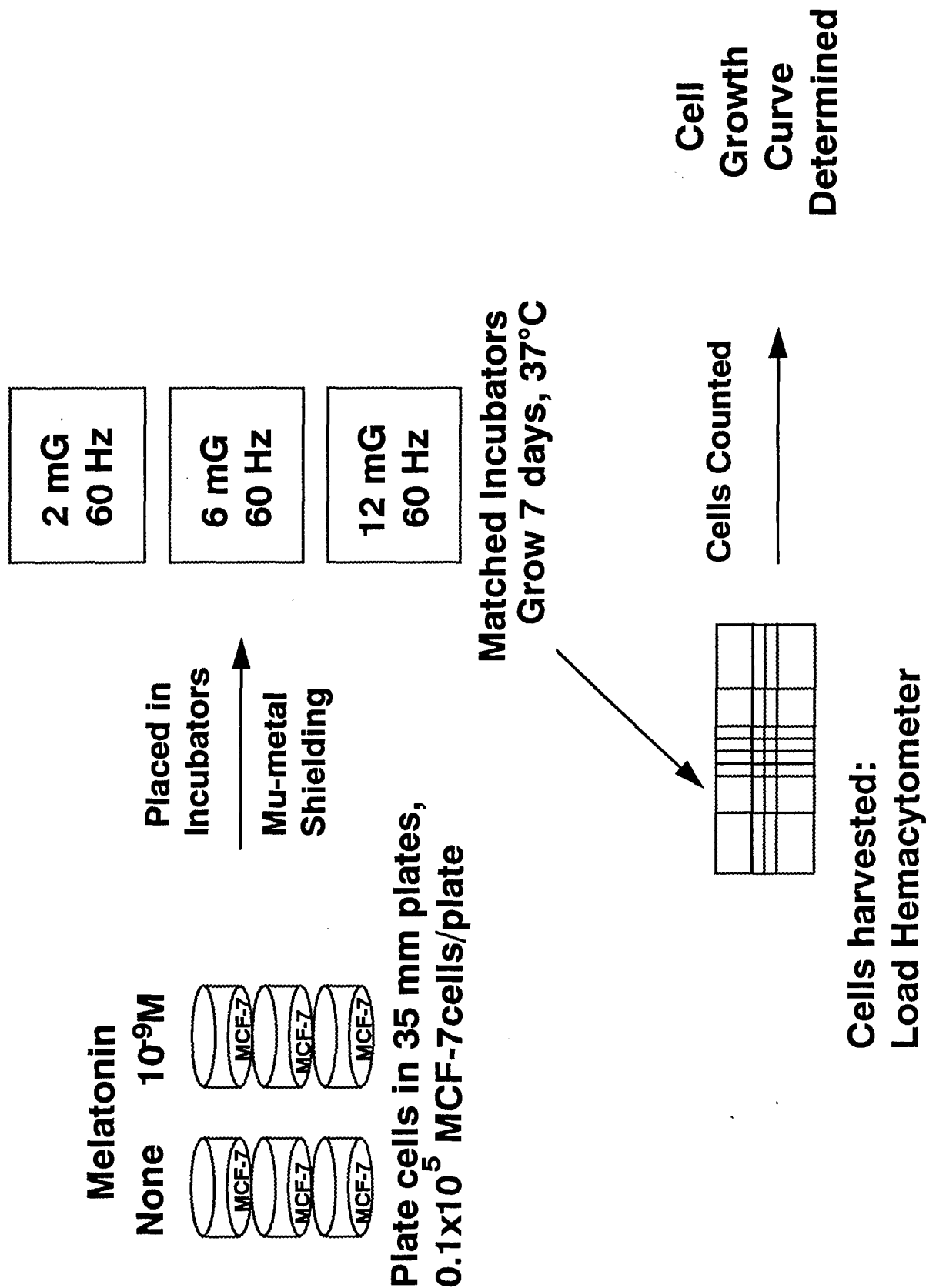
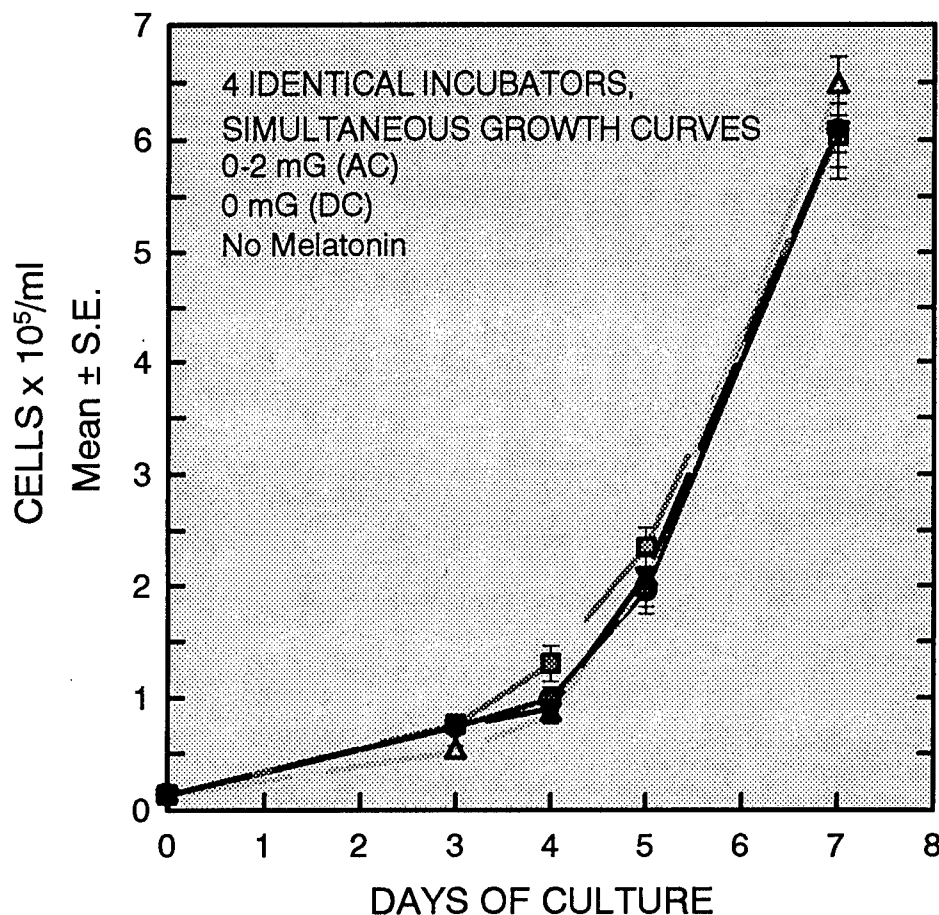


Figure 1

MCF-7 CELL GROWTH: COMPARISON ACROSS 4 IDENTICAL INCUBATORS



**WHEN SAME PASSAGE CELLS ARE CULTURED
IN MATCHED INCUBATORS, SIMULTANEOUSLY,
IDENTICAL EXPONENTIAL GROWTH CURVES
ARE OBTAINED.**

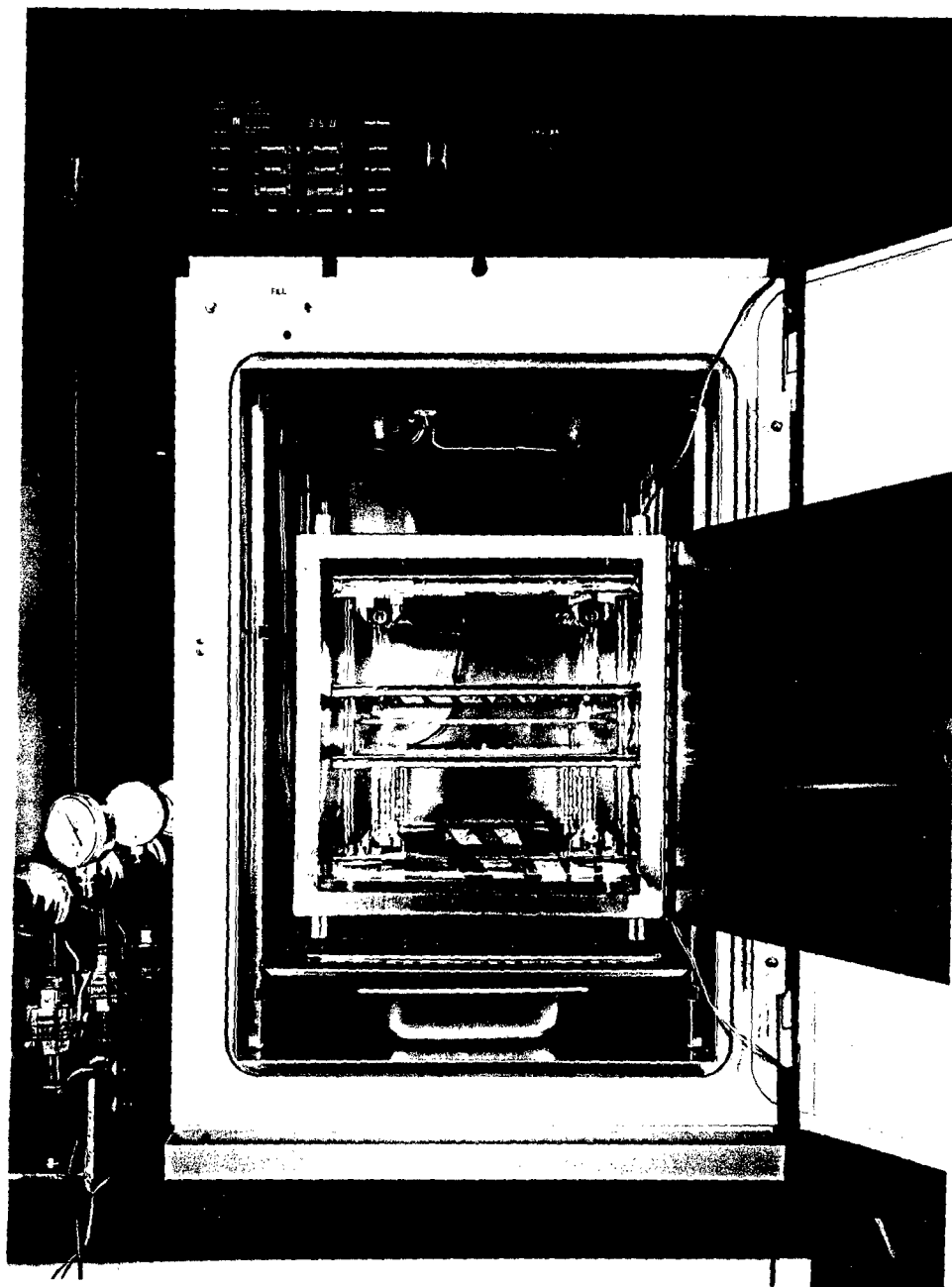
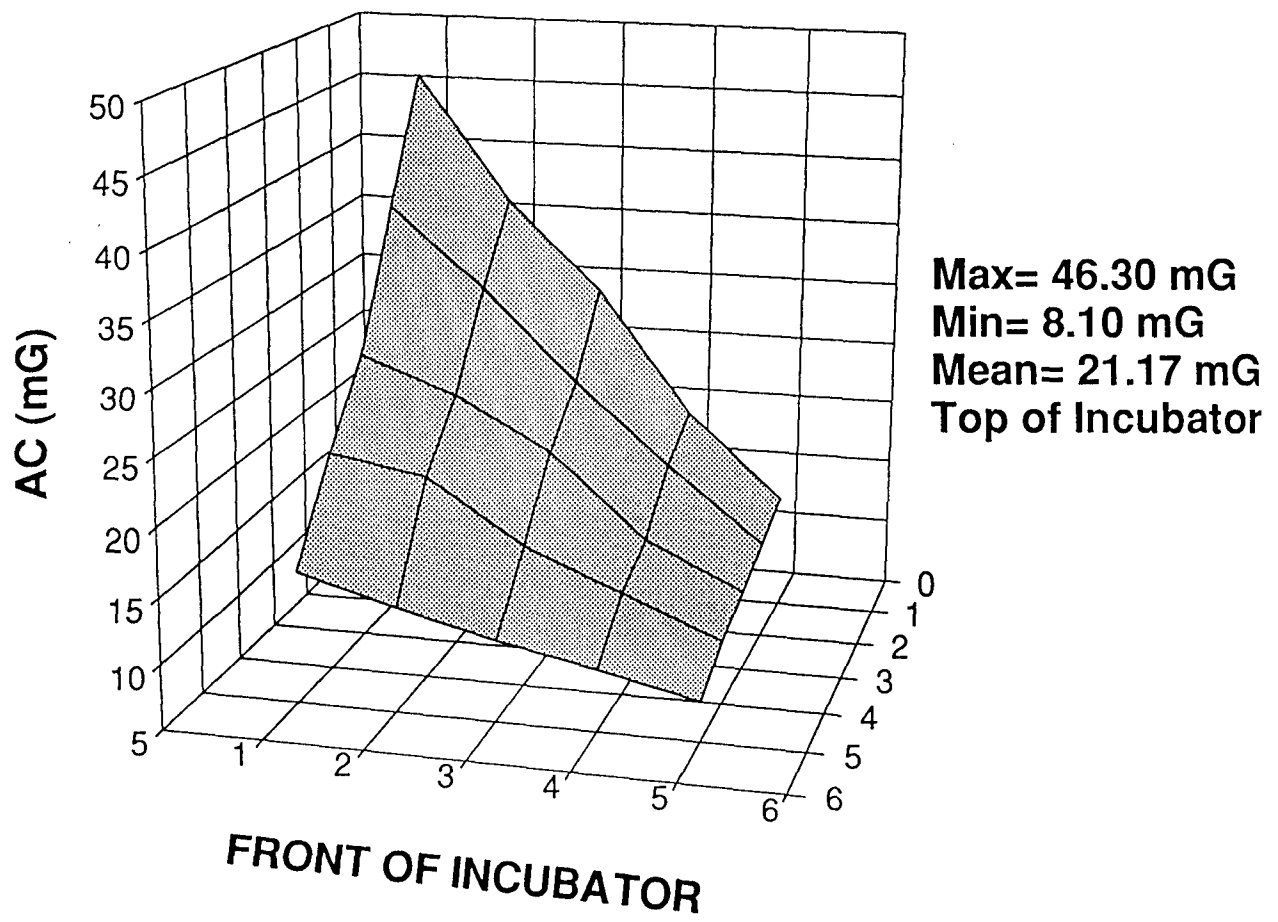
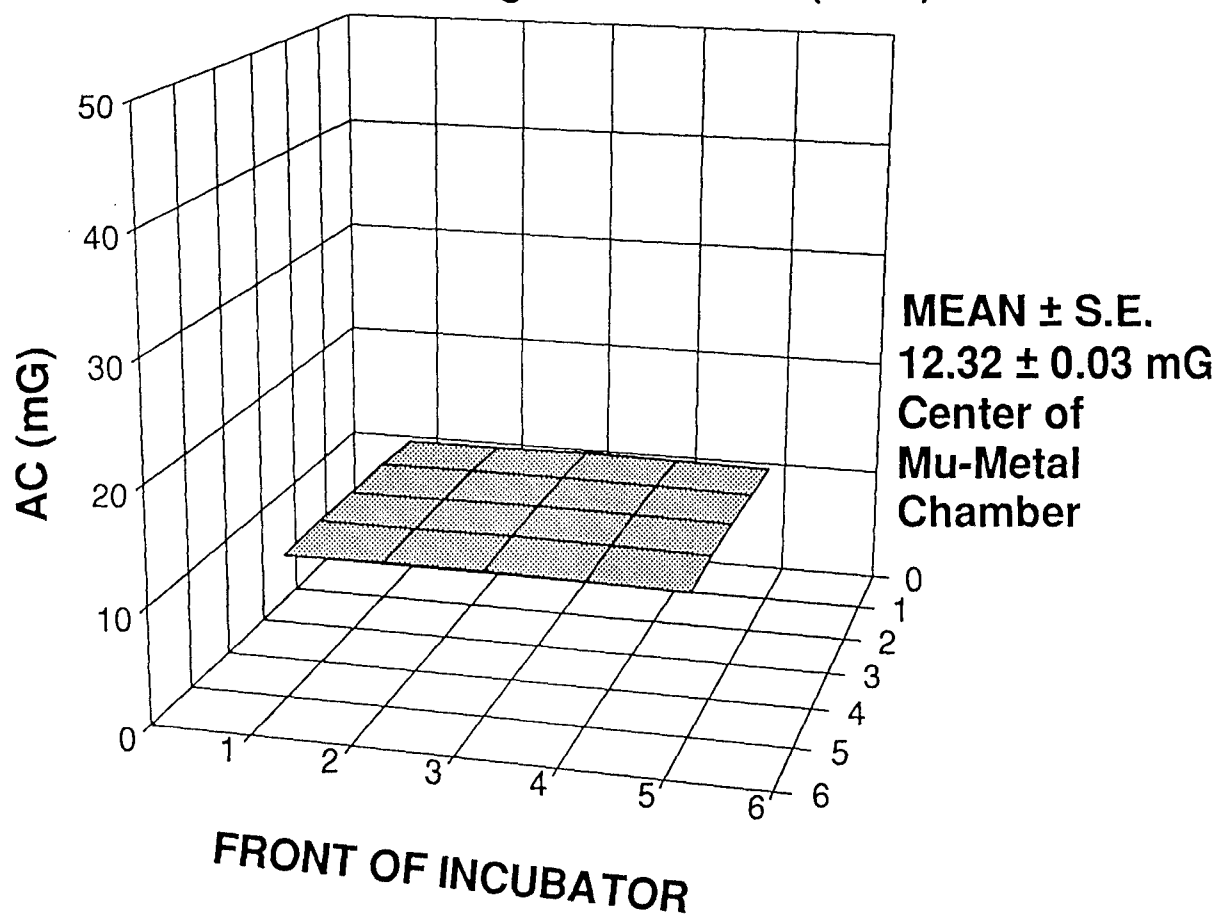


Figure 3

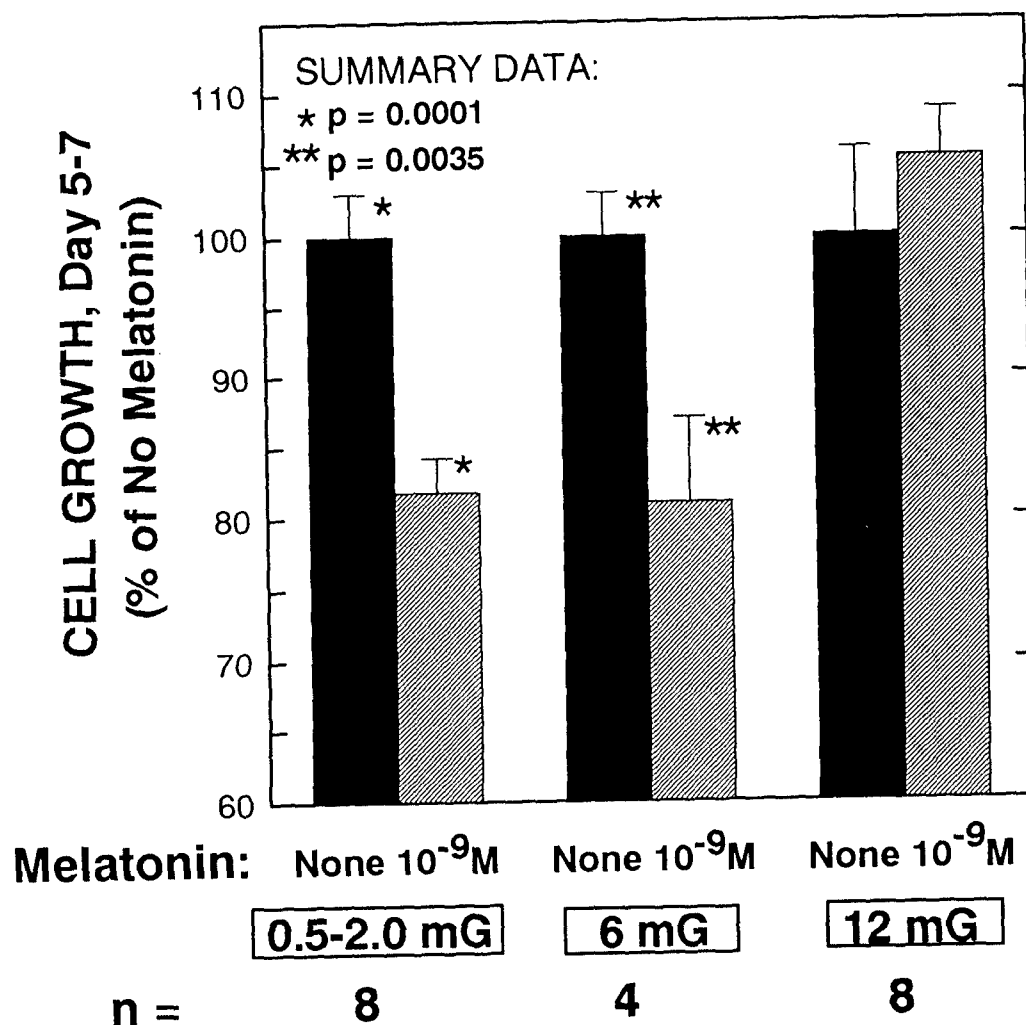
**Contour Map of AC Magnetic Fields Inside
Incubator Without Mu-Metal Chamber**



**Contour Map of AC Magnetic Fields Inside
Mu-Metal Chamber in Incubator:
Merritt Coil Energized at 12 mG (60Hz)**



12mG 60Hz MAGNETIC FIELDS BLOCK MELATONIN'S NATURAL ONCOSTATIC ACTION ON MCF-7 CELL GROWTH



**RESULTS INDICATE THAT A 12mG MAGNETIC FIELD BLOCKS
MELATONIN'S ONCOSTATIC ACTION.
A THRESHOLD EXISTS BETWEEN 6 - 12mG.**

We further investigated the dose-response relationship by conducting studies at higher field intensities to determine if the magnetic field blocks melatonin at fields as high as 1 Gauss. The same experimental approach of using identical passage cells across matched incubators, as discussed above, was used except that different field intensities were employed. **Figure 6** shows the results of these experiments. We observe that melatonin shows approximately 22% growth inhibition ($p < 0.003$) in a 2mG field, which is comparable to our previous observations. In addition, we observe a field blocking effect at 12mG ($p > 0.25$) and this is consistent with our previous observations. In the simultaneous exposures conducted at the higher field strengths of 20mG, and 1Gauss, we observe a field blocking effect, as well. These data suggest that the field blocking effect can be observed at fields as high as 1 Gauss.

Field Exposure Duration Studies.

We have addressed the question of exposure duration in a series of experiments. The design of these studies is shown in **Figure 7**. These experiments are referred to "timeshift" experiments, in that we conduct exposures to a 2 or 12mG field sequentially so that some cells get a continuous 2mG exposure for 7 days, while others receive 1, 2, or 5 days of exposure at 2mG and are then "shifted" into a 12mG field for the duration of the seven day growth period. With this experiment we addressed the question of critical exposure duration for the field blocking effect at 12mG.

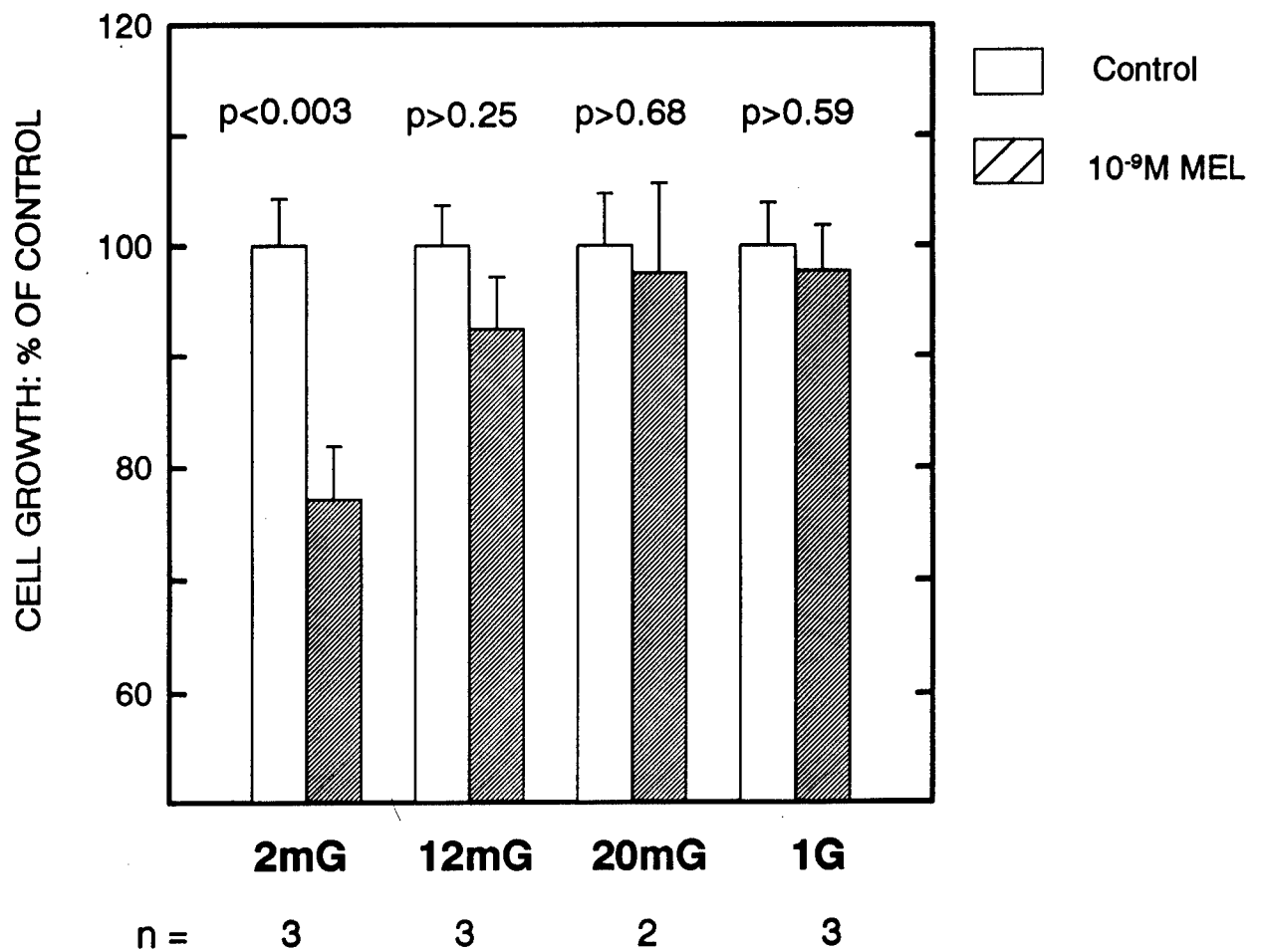
Figure 8 show the results from a series of experiments in which the above "timeshift" protocol was employed using tamoxifen at $10^{-7}M$. We observe that in a 2mG field on day 7 (far left-hand side bar graph) there is a ~30% growth inhibition due tamoxifen ($p < 0.05$). This level of growth inhibition is consistent with previous data from Year one. We observe the same level of growth inhibition for tamoxifen (~30%, $p < 0.05$) for cells that are maintained in a 2mG field for 1 or for 2 days, and then "switched" to a 12mG field for the rest of the 7 days growth curve (refer to bar graphs in **Figure 8**). When tamoxifen treated cells were left in 12mG field for 5 days, and then "switched" to a 2mG field, tamoxifen action was blocked. This blocking was indistinguishable from that observed for a 12mG exposure over all 7 days (far right-hand bar graph). Thus, this data suggests that a 12mG exposure is apparently required for at least the first 2 days of the 7 day growth curve for tamoxifen action to be blocked. The period of two days corresponds to one cell cycle period of growth for the MCF-7 cells, and this observation raises the question of whether the cell cycle is an important parameter in field exposure timing.

Phase II: Biologically-Based Studies.

We have addressed the question of whether steady-state levels of melatonin are altered in the growth media during field exposure in vitro. This question is important since if the concentration of melatonin in the media is altered over the growth cycle of MCF-7 cells maintained in a 12mG field, this might explain the field blocking effect. For example, if melatonin concentrations drop significantly in the media this might provide one possible explanation for a field blocking effect on melatonin action.

In these experiments we have cultured MCF-7 cells according to our standard protocol with or without melatonin present at $10^{-9}M$ [232 pg/ml] in a 2 or 12mG magnetic field. Cells were plated for a typical growth curve and we collected the media supernatant from dishes on days 1,2,3,4,5,6, and 7. The supernatant was immediately frozen and subsequently they were coded and shipped frozen to Dr. S. Yellon at Loma Linda University for melatonin analysis. Dr. Yellon has a RIA assay for determining melatonin concentrations in solutions [J. Pineal Res. (1994) 16:136]. For the determinations performed on these samples, the coefficients of variability

EFFECT OF FIELD DOSE ON MELATONIN'S CYTOSTATIC ACTION IN MCF-7 CELLS: DAY 7



Exposure Duration:

Tamoxifen Timeshift Experiment

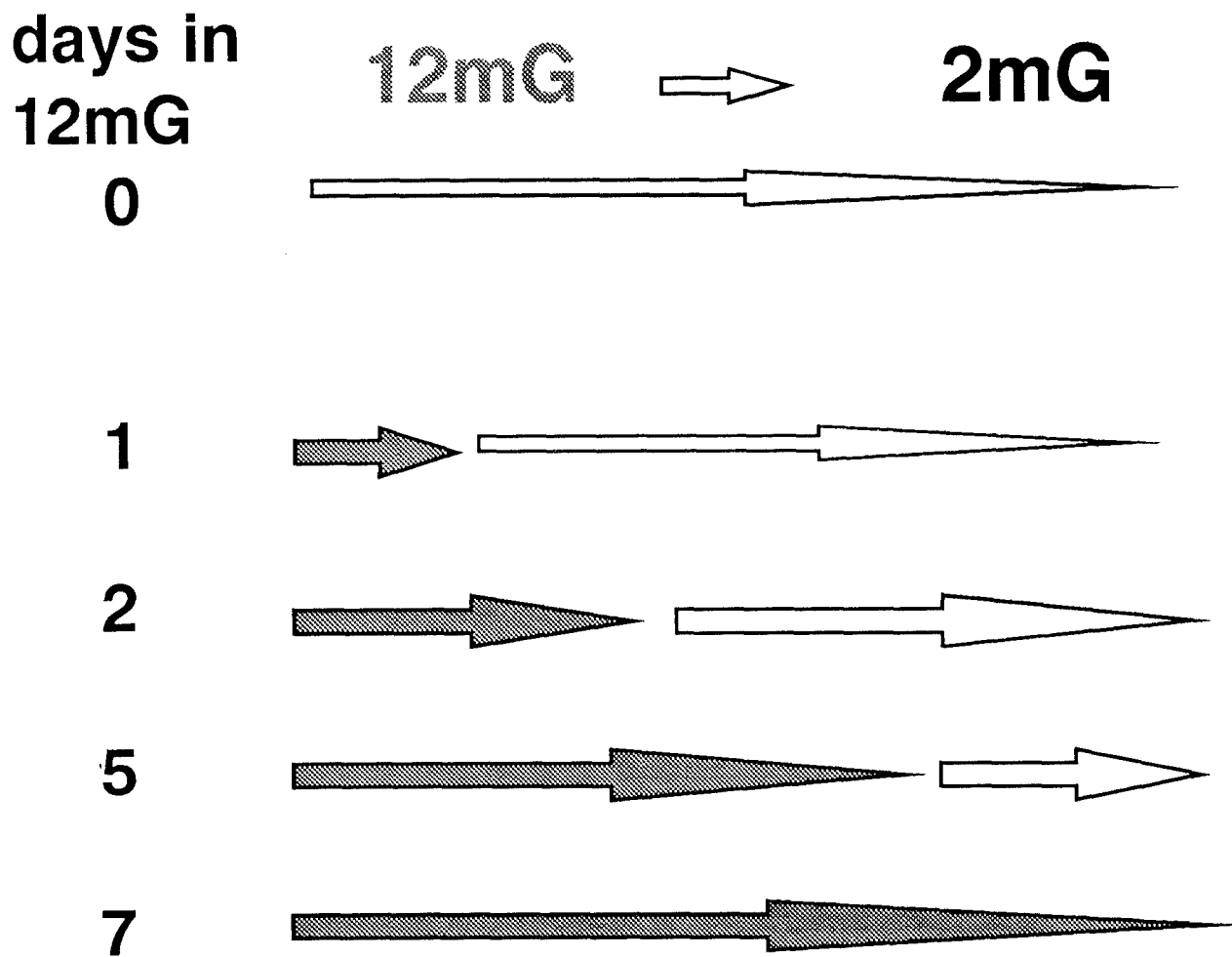
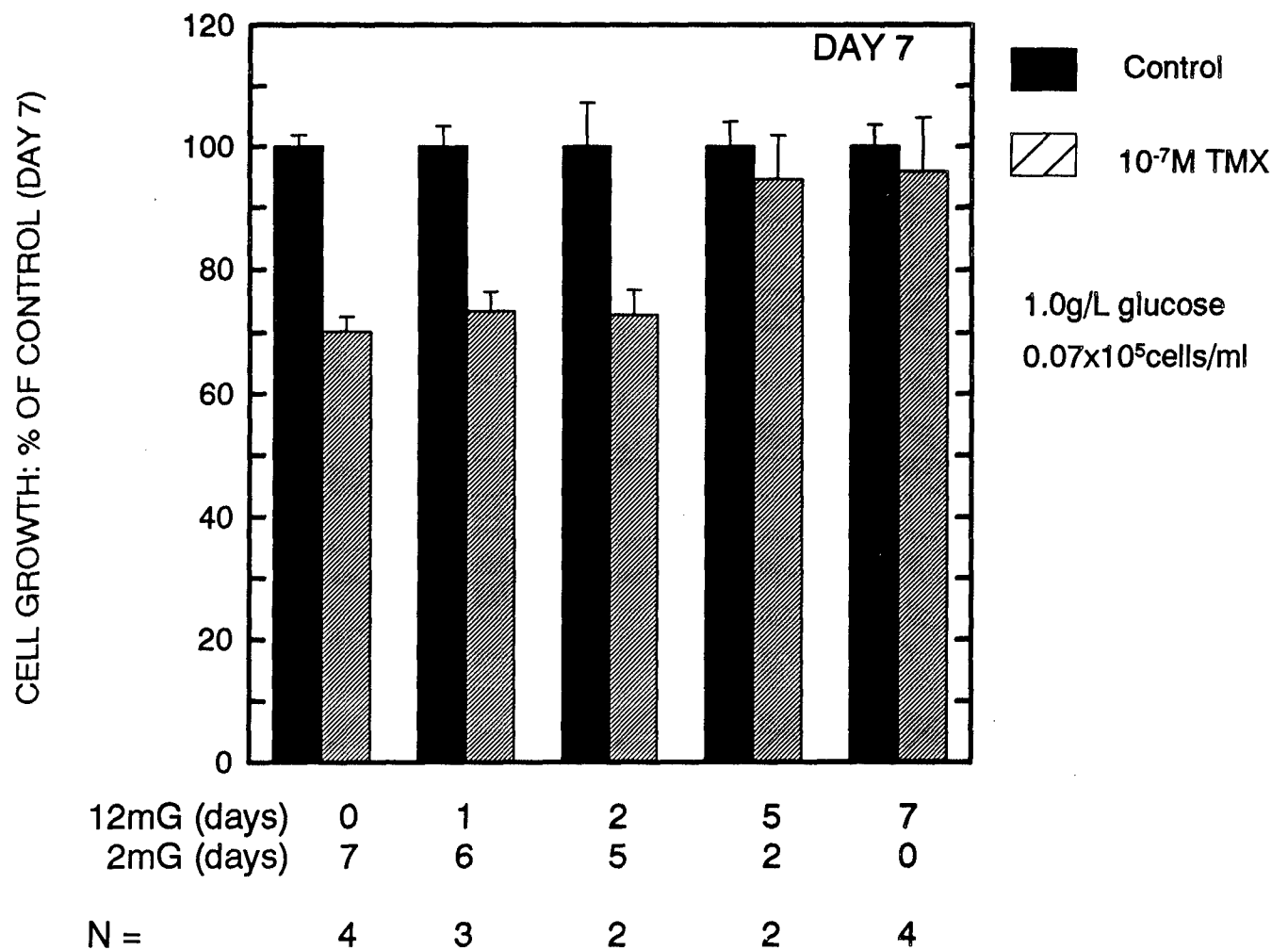


Figure 7

**EXPOSURE FOR ONE CELL CYCLE (2 DAYS) IS REQUIRED
FOR 12mG EFFECT**



between assays was 4.2% and within assays averaged 8.5%. The limit of assay sensitivity was 20 pg/ml.

The results are shown below as the average values across the seven day period given in pg/ml \pm S.E., n = 7(days). Melatonin levels within the three groups below did not vary significantly over the seven day period, so the overall means \pm S.E. for each group are shown.

	<u>Sample</u>	<u>2mG</u>	<u>12mG</u>
1.	Media(no cells)+Melatonin	249 \pm 17	253 \pm 11
2.	Media(no cells)+Melatonin+FBS	250 \pm 20	240 \pm 12
3.	Cell Supt.Media+ Melatonin+ FBS	303 \pm 12	303 \pm 11

The addition of FBS to the media (no cells present) did not significantly elevate melatonin levels (compare groups 1 vs. 2). The cell culture media during MCF-7 proliferation (group 3) remained relatively constant over a 7 days growth period for both 2 and 12mG. It appears from the data, as reflected in melatonin concentration in the media, that the availability of melatonin to cells is not significantly altered by the presence of the 2 vs. 12mG fields. These data suggest that variation in melatonin concentrations in cell culture does not occur as a result of field treatment. Therefore, alterations in melatonin concentrations in the media probably do not play a role in the 12mG blocking effect on melatonin action in MCF-7 cells.

Replication Studies at EPA.

Dr. Carl Blackman has independently replicated our original melatonin findings. He presented his data at the recent Bioelectromagnetics Society meetings, Victoria, CN, June 9-14, 1996. His abstract is attached. I note that he conducted these studies with MCF-7 cells from our laboratory, using our detailed protocols, and using the same cell culture media and serum as we use in our laboratory.

Dr. Blackman's results were published in his abstract and are provided below where the mean is times 10⁶ cells per ml. Cells were harvested and counted in a blinded manner.

	<u>Control</u>	<u>Melatonin</u>	<u>Melatonin & MF</u>
B Field	(<2mG)	(<2mG)	(12mG)
mean	1.38	1.15	1.39
S.E.	0.15	0.14	0.14
n	9	9	9

According to the statistical analysis presented in the abstract, the control and melatonin & MF treatments were not significantly different, but both means were significantly larger than the melatonin mean (p<0.001). These findings successfully replicate our original findings.

Conclusions.

There are several possible comments that can be made regarding the findings presented above.

1. We have continued to observe and to characterize the blocking or inhibition action of 12mG 60Hz, magnetic fields on the cytostatic function of both melatonin and tamoxifen in MCF-7 cells.

2. Magnetic field dose-response studies suggest that a field threshold may exist between 6 - 12mG for our melatonin findings.

3. The melatonin blocking effect we observe at 12mG is also observed at the higher field strengths of 20mG and at 1Gauss.

4. In exposure duration studies it appears that exposure to 12mG fields is required for at least two days for a field blocking effect on tamoxifen action. This suggest a possible role for the cell cycle in mediating the interaction.

5. In studies measuring melatonin concentrations in cell supernatants during 2 vs. 12mG field exposures that does not appear to be any significant alteration in melatonin availability to cells. Thus, the 12mG melatonin blocking effect is probably not due to nay alterations in melatonin availability in the cell media.

References.(Attached in Appendix in order shown below)

Abstracts Presented at National Meetings.

1. ELF Inhibition of Tamoxifen's Action on MCF-7 Cell Proliferation: Preliminary Mechanistic Studies. J.D. Harland and R.P. Liburdy. Presented at the 1994 Annual Review of Research on Biological Effects of Electric and Magnetic Fields From the Generation, Delivery, and Use of Electricity. Palm Springs, CA, November 12-16, 1995. Abstract A-3.

2. ELF Inhibition of Melatonin and Tamoxifen Action on MCF-7 Cell Proliferation: Field Parameters. J.D. Harland & R.P. Liburdy. Presented at the 18th Bioelectromagnetics Society meeting held on June 9-14 in Victoria, CN. Abstract A-1-1 (1996).

3. Melatonin Levels in Cell Culture Media During MCF-7 Cell Growth and Magnetic Field Exposures. S.M.J. Afzal, R.P. Liburdy and S. Yellon. Presented at the 18th Bioelectromagnetics Society meeting held on June 9-14 in Victoria, CN. Abstract P-7A (1996).

4. Independent Replication of the 12mG Magnetic Field Effect on Melatonin and MCF-7 Cells In Vitro. C.F. Blackman, S.G., Benane, D.E. House, J.P. Blanchard. Presented at the 18th Bioelectromagnetics Society meeting held on June 9-14 in Victoria, CN. Abstract A-1-2 (1996).

Appendix

1. ELF Inhibition of Tamoxifen's Action on MCF-7 Cell Proliferation: Preliminary Mechanistic Studies. J.D. Harland and R.P. Liburdy. Presented at the 1994 Annual Review of Research on Biological Effects of Electric and Magnetic Fields From the Generation, Delivery, and Use of Electricity. Palm Springs, CA, November 12-16, 1995. Abstract A-3.
2. ELF Inhibition of Melatonin and Tamoxifen Action on MCF-7 Cell Proliferation: Field Parameters. J.D. Harland & R.P. Liburdy. Presented at the 18th Bioelectromagnetics Society meeting held on June 9-14 in Victoria, CN. Abstract A-1-1 (1996).
3. Melatonin Levels in Cell Culture Media During MCF-7 Cell Growth and Magnetic Field Exposures. S.M.J. Afzal, R.P. Liburdy and S. Yellon. Presented at the 18th Bioelectromagnetics Society meeting held on June 9-14 in Victoria, CN. Abstract P-7A (1996).
4. Independent Replication of the 12mG Magnetic Field Effect on Melatonin and MCF-7 Cells In Vitro. C.F. Blackman, S.G., Benane, D.E. House, J.P. Blanchard. Presented at the 18th Bioelectromagnetics Society meeting held on June 9-14 in Victoria, CN. Abstract A-1-2 (1996).

PROJECT ABSTRACTS

**THE ANNUAL REVIEW OF RESEARCH ON BIOLOGICAL EFFECTS
OF ELECTRIC AND MAGNETIC FIELDS FROM
THE GENERATION, DELIVERY & USE OF ELECTRICITY**

PALM SPRINGS, CALIFORNIA, U.S.A.

NOVEMBER 12-16, 1995

Organized by the:

**U.S. DEPARTMENT OF ENERGY
OFFICE OF ENERGY MANAGEMENT**

**ELECTRIC POWER RESEARCH INSTITUTE
ENVIRONMENTAL DIVISION**

**U.S. ENVIRONMENTAL PROTECTION
AGENCY**

**NEW YORK STATE DEPARTMENT OF
PUBLIC SERVICE**

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

Co-Sponsored by:

**NATIONAL INSTITUTE FOR
OCCUPATIONAL SAFETY & HEALTH**

EDISON ELECTRIC INSTITUTE

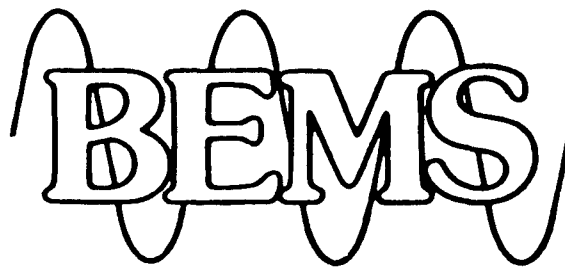
ELF INHIBITION OF TAMOXIFEN'S ACTION ON MCF-7 CELL PROLIFERATION: PRELIMINARY MECHANISTIC STUDIES. J.D. Harland and R.P. Liburdy. Cell and Molecular Biology Department, Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720, USA.

OBJECTIVE: We have begun studies to determine the mechanism(s) by which 12mG, 60 Hz fields block the inhibitory action of Tamoxifen on MCF-7 cell growth. First, we have accumulated evidence indicating that the B field is the operative metric. Secondly, we are determining the critical 12mG exposure time necessary to produce an irreversible blocking effect, indicating whether relatively fast (e.g., drug uptake) or slow (e.g., altered mRNA expression) biological processes are involved. Finally, since Tamoxifen is known to exert its effects by binding to the estrogen receptor (ER), we have begun studies determining whether the 12mG field acts by modulating cellular ER levels.

METHODS: We follow MCF-7 cell growth over seven days (seeding density: 0.1×10^5 cells/35mm plate), using identically matched incubators with mu-metal chambers enclosing 4-square Merritt coils. By rotating the 12mG field 90°, we reduce the E_{\max} field six-fold, which allows us to measure the relative contributions of the E and B field components. For determining critical exposure times, we count MCF-7 cells (control and Tamoxifen-treated) after exposure to a continuous 2mG field, a continuous 12mG field, or a short-term 12mG field followed by a 2mG field. For ER determinations, we quantitate cytosolic ER levels using a-ER monoclonal antibodies (ER-EIA kit, Abbott Labs), after MCF-7 exposure to either a 2mG or 12mG field for seven days.

RESULTS: Tamoxifen is the most widely used therapy for treatment of breast cancer, and is known to competitively bind the estrogen receptor. Recently, we have shown that a 12mG field can significantly reduce the growth inhibitory action of Tamoxifen ($10^{-7}M$) on MCF-7 cell growth. By comparing the effects of a vertical vs. a horizontal 12mG field, we have accumulated evidence that the field effects are most likely due to a B field interaction. In addition, we have begun preliminary experiments testing 1) the critical field exposure time necessary for blocking effects, and 2) whether the 12mG field exerts its effects by altering ER content of the cell. Preliminary experiments suggest that at least three days exposure at 12mG is necessary to irreversibly block the cytostatic action of Tamoxifen, indicating that prolonged 12mG exposure may be required. This result also raises the possibility of field effects that may be cell cycle dependent, since measurable effects appear to be delayed or reversible until cell division begins.

DISCUSSION: We plan to continue to characterize field parameters such as intensity threshold and frequency dependence, as well as investigate the ER as a possible site of interaction with the MF; both cytosolic and nuclear ER effects will be investigated. In addition, we will investigate whether any of the above effects are cell cycle dependent.



Elizabeth

Eighteenth Annual Meeting

Technical Program & Registration

**Conference Centre
Victoria, B.C., Canada**

June 9-14, 1996

22

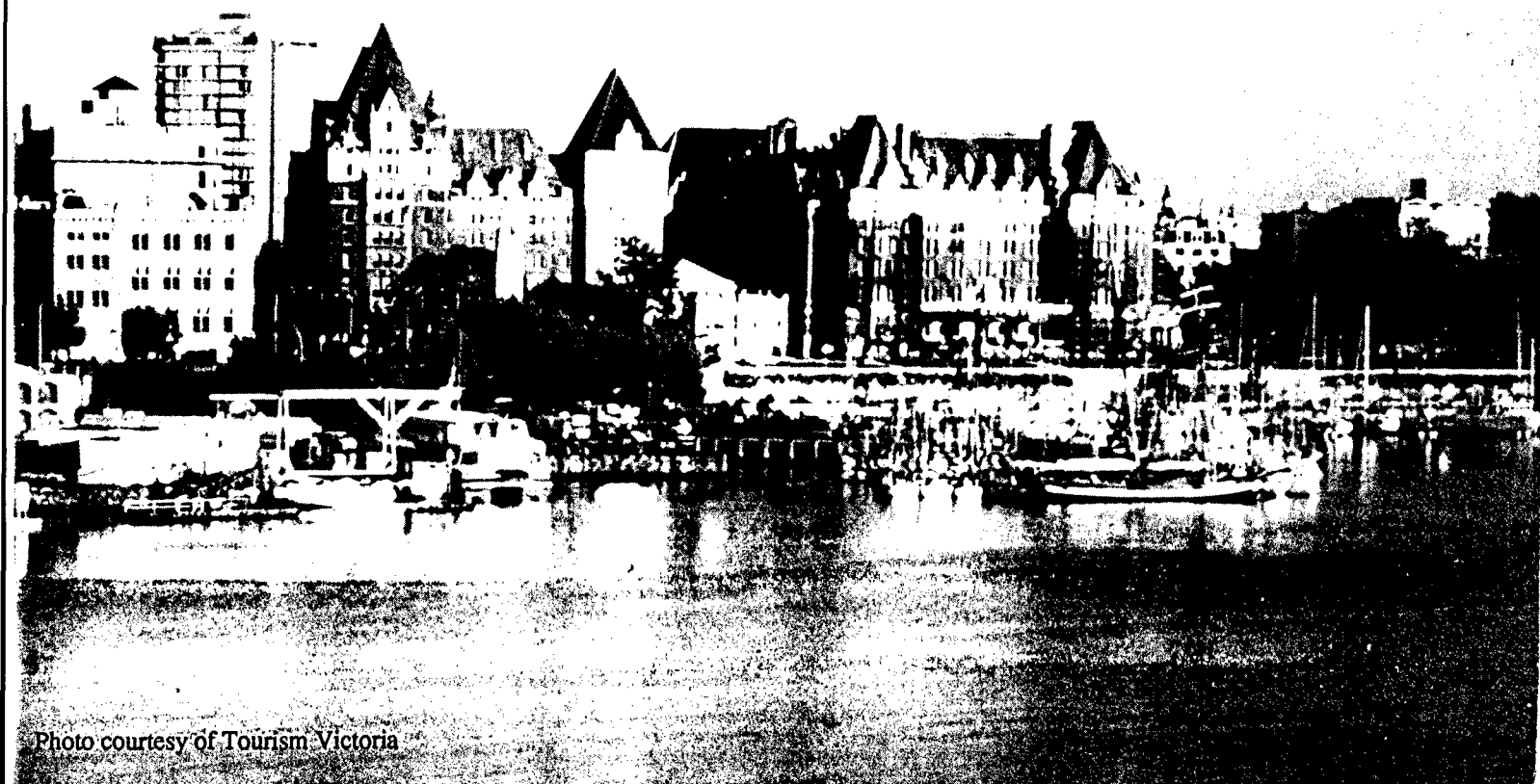


Photo courtesy of Tourism Victoria

A-1-1

ELF INHIBITION OF MELATONIN AND TAMOXIFEN ACTION ON MCF-7 CELL PROLIFERATION: FIELD PARAMETERS. J.D. Harland and R.P. Liburdy. Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720, USA.

OBJECTIVE: We have begun studies to define the parameters by which 12mG, 60 Hz fields block the inhibitory action of melatonin or tamoxifen on MCF-7 cell growth. (Tamoxifen is the most widely used therapy for treatment of breast cancer, and is known to competitively bind the estrogen receptor). We have accumulated evidence indicating that the B field is the operative metric [1]. More recently, we are determining the minimum duration of the 12mG exposure necessary to produce a blocking effect. Finally, we have begun studies determining the range of field magnitudes effective at blocking the hormone or drug. We are also collaborating with S. Engstrom to test his "fast/slow" hypothesis; see companion abstract.

METHODS: We follow MCF-7 cell growth over seven days (seeding density: 0.1×10^5 cells/35mm plate), using identically matched incubators with mu-metal chambers enclosing 4-square Merritt coils. By rotating the 12mG field 90°, we reduced the E_{max} field six-fold, which allowed us to measure the relative contributions of the E and B field components. For determining critical exposure times, we count MCF-7 cells (control and drug- or hormone-treated) after exposure to a continuous 2mG field, a continuous 12mG field, or a short-term 12mG field followed by a 2mG field. For field magnitude studies, we compare melatonin inhibition after MCF-7 exposure to a 2mG, 12mG, 20mG or 1 Gauss field.

RESULTS: We have shown that a 12mG field can significantly reduce the growth inhibitory action of melatonin (10^{-9} M) or tamoxifen (10^{-7} M) on MCF-7 cell growth [1]. By comparing the effects of a vertical vs. a horizontal 12mG field, we have provided evidence that the field effects are most likely due to a B field interaction. More recently, we have begun preliminary experiments testing 1) the critical field exposure time necessary for blocking effects, and 2) range of effective field magnitudes. Preliminary experiments suggest that at least three days exposure at 12mG is necessary to block the cytostatic action of tamoxifen (from 27% growth inhibition, $p < 0.0001$; to 5% growth inhibition, $p > 0.5$) indicating that prolonged 12mG exposure may be required. This appears to be consistent with a "slow" interaction mechanism. This result also raises the possibility of field effects that may be cell cycle dependent, since measurable effects appear to be delayed or reversible until cell division begins. In addition, all field magnitudes of 12mG or higher that have been tested thus far (12mG, 20mG, 1 Gauss) have been effective at blocking melatonin.

CONCLUSION: We plan to continue to characterize field parameters such as intensity threshold and frequency dependence, as well as investigate the ER as a possible site of interaction with the MF. In addition, we will investigate whether any of the above effects are cell cycle dependent.

[1] J. Harland and R. Liburdy (1995) BEMS Meeting, Boston, MA. Abstr. 3-3.

P-7A

MELATONIN LEVELS IN CELL CULTURE MEDIA DURING MCF-7 CELL GROWTH AND MAGNETIC FIELD EXPOSURES. S.M.J. Afzal¹, R.P. Liburdy¹ and S. Yellon². ¹Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA. ²Center for Perinatal Biology, School of Medicine, Loma Linda University, Loma Linda, California 92359, USA.

OBJECTIVE: Melatonin has been shown in several laboratories to significantly reduce the growth of MCF-7 cells in culture. D. Blask first reported this finding and we [reviewed in 1], and others, have confirmed this independently. To our knowledge the levels of melatonin present in cell culture media during the time course of these growth inhibition studies have not been measured. It is possible that as MCF-7 cells undergo cell cycle progression the "effective" concentration of melatonin in the media may change according to the dynamic growth activity of these cells. In addition, since we have previously reported that 12mG, 60Hz magnetic fields act to block melatonin's oncostatic action in cell culture, we have measured melatonin levels in the media of MCF-7 cells exposed to 2 or 12mG magnetic fields.

METHODS: MCF-7 cells were cultured in our laboratory as previously reported [1]. The experimental design involved using two mu-metal modified incubators operated at 2 or 12mG, 60Hz (DC fields negligible). Samples consisted of media alone, media plus melatonin, media plus FBS, media plus FBS plus melatonin, supernatant from cells containing FBS, supernatant from cells containing FBS and melatonin. When melatonin was present it was added at 10^{-9} M, which is 232 pg/ml. Samples were collected on days 0, 1, 2, 3, 5, 6, and 7 and were immediately frozen for storage. Samples were shipped frozen from LBL to Loma Linda and assayed blinded and in duplicate, for melatonin levels according to protocols used at Loma Linda [*J. Pin. Res.* (1994) 16; 136]. Coefficients of variability between assays was 4.2% and within assays averaged 8.5%. The limit of assay sensitivity was 20 pg/ml.

RESULTS: First it was determined that the presence of FBS in the frozen samples did not contribute to melatonin levels. FBS contributed <20pg/ml which is the limit of sensitivity of the assay. For each sample treatment little or no change was observed as a function of collection days. Melatonin concentrations (Mean \pm S.E., n=7) were obtained for samples across collection days.

	2 mG	12 mG
Media + Melatonin	249 \pm 17 ^a	253 \pm 11 ^c
Media + Melatonin + FBS	250 \pm 20 ^a	240 \pm 12 ^c
Cell Supt. + Melatonin + FBS	303 \pm 12 ^b	303 \pm 11 ^d

data with different superscripts (a vs.b and c vs.d) are significantly different, $p < 0.02$

CONCLUSION: Melatonin levels in cell culture media during MCF-7 proliferation remain relatively constant over a 7 day growth period. In addition, it appears from the data that the availability of melatonin, as reflected in melatonin concentration in the media, is not significantly altered by the presence of a 2 or 12 mG sinusoidal 60Hz magnetic field. These data suggest that variation in melatonin concentrations in cell culture do not occur in the absence or in the presence of these magnetic fields. Therefore alterations in melatonin concentration in the media probably do not play a role in our previously reported magnetic field effects on MCF-7 cell growth [1].

[1] R.P. Liburdy (1995). *Radio Science* 30; 179-203.

A-1-2

INDEPENDENT REPLICATION OF THE 12-MG MAGNETIC FIELD EFFECT ON MELATONIN AND MCF-7 CELLS IN VITRO. C.F. Blackman¹, S.G. Benane^{2*}, D.E. House^{2*} and J.P. Blanchard¹. ¹National Health & Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, USA. ²Bechtel Corporation Research & Development, San Francisco, California 94119, USA.

Recent studies showed that physiological concentrations of melatonin (10^{-9} M) reduce the growth rate of transformed human breast cancer (MCF-7) cells in culture (1,2), but 12-mG, 60-Hz magnetic fields eliminate this melatonin-induced growth rate inhibition, while 2-mG, 60 Hz magnetic fields have no such effect (2,3).

OBJECTIVE: With the cooperation of the originating laboratory, our goal was to independently replicate these melatonin and magnetic field effects on MCF-7 cells (2).

APPROACH AND METHODS: We carefully followed the detailed protocol provided by the original laboratory which included using the same cells and lot of serum as in the original report. Mu-metal chambers screened ambient magnetic fields during treatments, allowing careful control of all fields to which the cells were exposed during testing. There were two deviations from the original protocol: a) our growth of stock MCF-7 cells was in a magnetically unshielded incubator, although the ambient 60-Hz fields were ≤ 2 mG as required by the protocol, and b) we used Helmholtz rather than Merritt coils to generate the magnetic fields during our tests. Three replications of each test were performed and each experiment employed a newly thawed vial of MCF-7 cells. Three dishes of cells were plated for each of three treatment conditions created in mu-metal boxes housed in 5% CO₂ incubators, as described below. Each treatment was continuous for 7 days. Cells were then harvested and counted in a blinded manner.

RESULTS: Combined results of the three experiments are:

	Control	Melatonin(MEL)	Melatonin(MEL) & MF
B Field	(<2 mG)	(<2 mG)	(12 mG)
mean	1.38	1.15	1.39
SE	0.15	0.14	0.14
n	9	9	9

where the mean is times 10^6 cells per ml. These results were analyzed by the REGWF multiple comparison procedure, the results of which indicated that the control and MEL & MF treatment means were not significantly different, but both means were significantly larger ($p < .001$) than the MEL mean.

DISCUSSION: These results independently confirm that a) melatonin can inhibit the growth of MCF-7 cells in culture (1), and, b) a 12-mG, 60-Hz magnetic field can completely block this oncostatic action (2). These results are particularly significant because: a) we believe our findings represent the first replication of a key magnetic field-induced bioeffect, and b) this foundation allows theorists to generate "testable" hypotheses to shed light on interaction mechanisms, both physical and biological in nature, using MCF-7 cell-based experimental data. The constructive communication established between our lab and the original lab lead to our ability to independently replicate their findings, a result which plays a critical role in scientific progress.

(1) Hill & Blask (1988) *Cancer Res.* 48:6121-6126.

(2) Liburdy, et al. (1993) *J. Pineal Research* 14: 89-97.

(3) Liburdy, et al. (1994) BEMS Mtg, Copenhagen, Abst. F-1-7.

This abstract does not reflect EPA policy. Partial support for this work is through an interagency agreement with the Department of Energy, Office of Energy Management, IAG# DE-AI01-94CE34007.